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Genetic analyses of archival specimens of the Atlantic sturgeon *Acipenser sturio* L., 1758

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ABSTRACT

Genetic variability was analysed in *Acipenser sturio* L., 1758 and *Acipenser oxyrinchus oxyrinchus* Mitchill, 1815 using variation in the D-loop region of mtDNA and a number of microsatellites (nuclear markers). The studied material included tissue samples from: (1) 38 *A. sturio* archival specimens collected in different German, Swedish, Danish, and French museums of natural history; (2) 27 live *A. sturio* representing a broodstock for restoration of this species in German waters (Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany); (3) 30 wild *A. o. oxyrinchus* caught in the Atlantic Ocean near the coast of New Jersey and in the Delaware River (USA); and (4) 60 individuals of *A. o. oxyrinchus* from an artificially reproduced stock originally obtained from several wild sturgeons captured in the St. John River (Canada). A 250-bp fragment of the D-loop region of mtDNA was cloned and sequenced. The length of a repeated unit was 80 bp in *A. sturio* and 79 bp in *A. o. oxyrinchus*. The repeated units of *A. sturio* and *A. o. oxyrinchus* differed by 11 substitutions and one deletion or insertion, respectively. No heteroplasmy was found. Three different haplotypes of mtDNA were observed in both species. Five microsatellites had polymorphic band patterns. In *A. sturio*, analyses of microsatellites showed a decrease in allelic numbers between the years 1823 and 1992. This decline resulted in a fixation of several alleles. For *A. sturio,* one mtDNA haplotype and seven alleles were observed only in archival samples. Genetic distance calculations showed a great genetic similarity between *A. sturio* populations in the Gironde River and the North Sea, and a basal position of the Mediterranean and Adriatic Sea *A. sturio* populations. In *A. o. oxyrinchus*, the number of microsatellite alleles ranged between 14 (Hudson and St. John rivers) and 22 (Delaware River). Genetic distance calculations showed a high genetic similarity between subpopulations of *A. o. oxyrinchus*.

Key words: *Acipenser oxyrinchus oxyrinchus,* breeding programme, genetic drift, restoration.

RESUMEN

Análisis genéticos de ejemplares almacenados de esturión atlántico* Acipenser sturio *L., 1758

Se analizó la variabilidad genética en Acipenser sturio *L., 1758 y* Acipenser oxyrinchus oxyrinchus *Mitchill, 1815 usando la variación en la región D-loop del ADN mitocondrial y en un número de microsatélites (marcadores nucleares). El material estudiado incluyó muestras de tejidos de: (1) 38 ejemplares almacenados de* A. sturio *colectados en diferentes museos de historia natural alemanes, suecos, daneses y franceses; (2) 27* A. sturio *vivos correspondientes al stock de cría para recuperación de esta especie en aguas alemanas (Instituto de Ecología Dulceacuícola y Pescas Interiores, Berlín, Alemania); (3) 30* A. o. oxyrin-

chus *silvestres capturados en el océano Atlántico cerca de la costa de Nueva Jersey y en el río Delaware (USA); y (4) 60 individuos de* A. o. oxyrinchus *de un stock reproducido artificialmente obtenido originalmente de diversos esturiones silvestres capturados en el río San Juan (Canadá). Se clonó y secuenció un fragmento de 250 pares de bases de la región D-loop del ADN mitocondrial. La longitud de una unidad repetida fue de 80 pares de bases en* A. sturio *y de 79 en* A. o. oxyrinchus. *Las unidades repetidas de* A. sturio *y* A. o. oxyrinchus *difirieron por 11 sustituciones y una delección o inserción, respectivamente. No se encontró heteroplasmia. Se observaron tres diferentes haplotipos de ADN mitocondrial en ambas especies. Cinco microsatélites presentaron patrones polimórficos de bandas. En* A. sturio *los análisis de microsatélites mostraron una disminución en números alélicos entre los años 1823 y 1992. Este declive tuvo como consecuencia la fijación de varios alelos. Para* A. sturio*, se observaron un haplotipo de ADN mitocondrial y siete alelos sólo en los ejemplares almacenados. Los cálculos de distancia genética mostraron una gran similitud genética entre las poblaciones del Gironda y del Mar del Norte, y una posición basal de las poblaciones de* A. sturio *del Mediterráneo y del Adriático. En* A. o. oxyrinchus *el número de alelos de microsatélite varió entre 14 (ríos Hudson y San Juan) y 22 (río Delaware). Los cálculos de distancia genética mostraron una gran similitud genética entre las subpoblaciones de* A. o. oxyrinchus.

Palabras clave: Acipenser oxyrinchus oxyrinchus*, programa de cría, deriva genética, recuperación.*

INTRODUCTION

Knowledge of the dynamics of gene flow, genetic drift, and selection in natural sturgeon populations on a spatial and temporal scale plays an important role in recent and future conservation plans. A large literature exists on the genetic differentiation at the species level for extant sturgeons (Birstein, Betts and DeSalle, 1998; Birstein *et al.*, 1998; Birstein and DeSalle, 1998). To date, only limited data were available for archival samples taken from museum specimens. Ferguson and Duckworth (1997) included three specimens of *Acipenser fulvescens* Rafinesque, 1817 from the Natural History Museum in London in their study of this species's genetic structure. These specimens were collected between 1866 and 1873. Until now, the genetic analysis of sturgeons has focused on phylogenetic reconstructions and species identification (Birstein, Hanner and DeSalle, 1997; Birstein, Betts and DeSalle, 1998; Birstein *et al.*, 1998; Birstein and DeSalle, 1998). Only few data regarding the genetic structure of the European Atlantic sturgeon *Acipenser sturio* L., 1758 have been published (Wirgin, Stabile and Waldman, 1997; Ludwig and Kirschbaum, 1998; review in Birstein and Doukakis, 2000). However, recent developments of molecular methods in general and the PCR techniques in particular have opened up the possibility of studying DNA from museum specimens (Nielsen, Hansen and Loeschcke, 1997, 1999). Thus, information important for conservation programmes, such as the historic gene flow, migration routes, genetic distances, and subpopulation structure, can be obtained by using archival samples.

The objective of our study was to establish a method for the investigation of archival samples in order to analyse genetic variability and similarity among several extinct stocks of *A. sturio,* as well as to characterise the German broodstock that has been created at the Institute of Freshwater Ecology and Inland Fisheries (IFEIF), Berlin. Also, we wanted to assess the influence of population decline on the gene pool of *A. sturio*. Since sequence differences are low in several mitochondrial (mt) genes among sturgeon species (Birstein, Hanner and DeSalle, 1997), we studied microsatellites in addition to the partial sequence of the D-loop. In fishes, as in other animals, the D-loop is the most variable region of the maternally inherited mtDNA, whereas microsatellites are known as highly polymorphic nuclear markers (Meyer, 1993). Also, we performed a similar study on three populations of *Acipenser oxyrinchus* Mitchill, 1815, the American Atlantic sturgeon, which is a sister species to *A. sturio* (Birstein and Bemis, 1997), and compared data for these two species.

MATERIALS AND METHODS

Samples

We collected archival samples from 44 specimens of *A. sturio* kept in different German, Swedish, Danish, and French natural history museums (table I). Only limited data are available regarding the collection and storing conditions of these specimens. For DNA extraction, we took different tissue samples from each fish: fin clips, pieces of gill arches and/or of skin. Since histori-

cally the North Sea and its tributaries were the most important areas for sturgeon reproduction in German waters (Holčík *et al.*, 1989; Debus, 1995), we mainly used specimens collected in the North Sea or the Elbe River (table I). However, we also included two specimens from the Mediterranean and

Table I. Names of museum collections, and the location and year of the catch of archival specimens included in the present study. (*): Specimens from which the mtDNA sequences were successfully amplified. ([1]): BS = Baltic Sea, NS = North Sea, and MM = Mediterranean Sea. ([2]): The sign (<) before the date means that the sturgeon was caught before this date but included in the collection during this year. ([3]): This specimen came from the artificially reproduced *A. sturio* progeny of sturgeons caught in the Elbe River (Germany); a series of successful artificial reproduction of *A. sturio* was carried out in the town of Glückstadt, near Hamburg, between 1886 and 1891 (Mohr, 1952); no successful artificial reproduction of this species was reported after 1891; this specimen was about three days old, and it was included in the MfN collection in Berlin in 1903

Collection	Museum number	Location of the catch[1]	Year of the catch[2]
National Natural History Museum (MNHN), Paris	4634	Russia, unknown location	< 1870
	4636	Russia, unknown location	< 1870
	4637	Russia, unknown location	< 1870
	4638	Europe, unknown location	< 1870
	5158	NS, Elbe River	< 1870
	5165 *	MM, Tiber River	1823
Natural History Museum (MfN), Berlin	No number (a juvenile) [3]*	NS, Elbe River	1903
Koenig Museum, Bonn	8 *	NS, Helgoland Island	1993
Natural History Museum (NRM), Stockholm	1709	BS, Soedermanland	1890
	1837	NS, Bohuslän	1837
	13336 *	NS, Kattegeatt	1991
	18853	NS, Elbe River	1885
	35437	BS, Tosteberga	1871
	36001	NS, Hanstholmen Island	1985
	21705	NS, Bohuslaen	1838
	21706	NS, Bohuslaen	1897
	21707	BS, Soedermanland	1932
	21708	NS, Stroemstad	1910
	21710	NS, Fjaellbacka	1895
Rostock University (Germany)	A2	NS, Elbe River	1903
	A4	NS, Dutch coast	1992
	A14	BS, Warnemünde	1885
	A15	BS, Rostock	1883
	A16	BS, Warnemünde	1887
	A17	BS, Rostock	1882
	A18	BS, Rostock	1887
Biological Institute Helgoland (Germany)	E 01 *	NS, Elbe River	1959
	E 02 *	NS, Helgoland Island	1991
	E 03 *	NS, Helgoland Island	1988
Senckenberg Institute (MF), Frankfurt/Main	MF 7647 *	Adriatic Sea	1827
Zoological Museum, Dresden	*	Elbe River, near Dresden	1871
Zoological Museum, Hamburg	10476	NS, Oste River	1903
	10477	NS, Oste River	1903
	88	NS, Oste River	1907
Copenhagen University Zoological Museum (ZMUC), Copenhagen	42	BS, Copenhagen	1873
	CN16	BS, Copenhagen	1940
	P10202 *	NS, Helgoland Island	1956
	P10218 *	NS, Hirtshals	1986

Adriatic seas (MF 7647 and MNHN 5165, respectively; see table I).

Additionally, 27 individuals representing the German broodstock kept at the IFEIF for restoration of *A. sturio* in German waters were investigated. These originated from artificial reproduction of several specimens from the French stock obtained at the National Agricultural and Environmental Engineering Research Centre (Cemagref) in Bordeaux (France) from two individuals, a female and a male, caught in the Gironde River system in France in 1995 (Anon. 1995; Williot *et al.*, 1997).

Also, 90 specimens of the North American *Acipenser oxyrinchus oxyrinchus* were included in our study for comparison with *A. sturio*. Specimens of *A. o. oxyrinchus* were obtained from three different populations on the North American Atlantic coast: (1) 14 individuals were caught in the Atlantic Ocean near the town of Belmar (New Jersey, USA), close to the mouth of the Shark River –most likely, these sturgeons belonged to the Hudson River population (Waldman and Wirgin, 1998) and we will consider them as such; (2) 16 sturgeons were collected in the Delaware River (New Jersey, USA; see Secor and Waldman (1999) on the history of this population); and (3) 60 sturgeons were obtained from an artificially reproduced stock at the Sturgeon Conservation Centre in New Brunswick (Canada). Several breeders caught in the St. John River (Canada) were used to create this stock. These three populations represented *A. o. oxyrinchus,* one of the two *A. oxyrinchus* subspecies, the other being *A. oxyrinchus desotoi* Vladykov, 1955 (Waldman and Wirgin, 1998).

DNA extraction

Twenty-five µg tissue from each sample was washed in 0.68 % NaCl solution for 24 h at 4 °C, then homogenised and transferred to 180 µl Lysis Buffer (QIAGEN, Germany). After the complete lysis, DNA was extracted and washed following the QIAamp Tissue Kit (QIAGEN, Germany) standard protocol. The DNA concentration was estimated using the DipStick Kit (Invitrogen, Netherlands).

MtDNA amplification and sequencing

Traditionally, PCR fragments between 1.6 and 2.2 kb are used for the detection of heteroplasmy in sturgeons (Brown, Beckenbach and Smith, 1992; Brown *et al.*, 1996; Miracle and Campton, 1995). Taking into consideration the difficulties of amplification of long PCR fragments when studying archival samples, we used two primers closely located to the repeated region, Hetero I (5´-ACCCTTAACTCCCAAAG-3´) and Hetero II (5´-CATTTRATGGTAGATGAAAC-3´) (Ludwig and Jenneckens, 2000).

PCR amplification was performed in a total reaction volume of 25 µl containing approximately 10 ng template DNA, 2.5 µl 10× reaction buffer, 10 pmol of each primer, Hetero I and Hetero II (Ludwig and Jenneckens, 2000), 100 mM dNTPs, 2.5 mM $MgCl_2$, and 0.5 units *Taq* DNA polymerase (Oncor-Applegene, Germany) under the following reaction conditions: 94 °C, 20 s; 50 °C, 10 s; and 72 °C, 1 min with a final elongation step at 72 °C for 4 min. PCR products were separated on a 2.0 % agarose gel at 150 V for 2 h.

Potentially, different factors can influence the results of PCR reaction in the case of archival samples. Numerous examples of artefacts and pseudogenes have been described which included several mitochondria genes (Van der Kuyl *et al.*, 1995; Zhang and Hewitt, 1996). Sampling methods and changes in storing conditions of the selected museum specimens resulted in contamination. Thus, eight different sequences were observed from a single PCR amplification using DNA extracted from museum samples (Van der Kuyl *et al.*, 1995). To minimise these factors, we repeated all analyses twice and cloned PCR products before sequencing. In addition, sequencing analyses were done in two different labs: at the IFEIF (Berlin) and at the Institute of Animal Breeding and Genetics, University of Göttingen (Germany).

For sequencing the amplified heteroplasmic fragments, PCR products were extracted from agarose gel, purified with QIAquick Gel Extraction Kit (QIAGEN, Germany), and cloned with TOPO TA Cloning Kit (Invitrogen, Netherlands) using standard conditions. Ten clones of each sample were amplified in 50 µl reaction volumes containing 2.5 units AmpliTaq DNA Polymerase (ABI, USA), 10 pmol primer (Hetero I and Hetero II), and 25 ng DNA of each sample. The reaction was amplified in a Perkin Elmer thermocycler 2400 programmed for 30 cycles each for 94 °C at 10 s; 50 °C at 10 s; and 72 °C for 2 min. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Germany). Sequencing was performed in 20 µl reaction volume con-

taining 3.5 µl BigDye RR Terminator Cycle Sequencing Kit (ABI, USA), 10 pmol primer and 15 ng DNA, and amplified in a Perkin Elmer thermocycler 2400 programmed for 25 cycles each for 94 °C at 10 s; 50 °C at 10 s, and 60 °C for 4 min. PCR products were sequenced in an ABI 310 automated sequencer (ABI, USA).

Statistical analysis of mt sequences

The 250-bp region of the $tRNA^{Pro}$ and the D-loop sequences were used for calculation of p-distance values (Kumar, Tamura and Nei, 1993). Using the computer package MEGA (Kumar, Tamura and Nei, 1993) and the p-distance outcomes, we constructed a neighbour-joining tree (Saitou and Nei, 1987). Bootstrap analyses (500 steps) were also performed in MEGA.

Microsatellites

Genetic variability was screened using 11 primer pairs named Afu-19, Afu-22, Afu-23, Afu-34, Afu-39, Afu-54, Afu-57, Afu-58, Afu-62, Afu-68, and Afu-69 (May, Krueguer and Kincaid, 1997). Primers were labelled with 6-FAM and TET (ABI, USA). Amplifications were performed in a total volume of 25 µl containing 50 ng genomic DNA, 0.25 U *Taq* polymerase, 5 pmol primers, 0.10 mM Tris-HCl (pH 8.8 at 25 °C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 µg/µl bovine serum albumin (BSA), 0.08 % (v/v) Nonidet P40, and 100 µM of each dNTP. Amplification was performed with 40 cycles of the following steps: 30 s at 94 °C, 30 s at 57 °C (Afu-34, 39, and 68) or 52 °C (Afu-19 and 54), 30 s at 72 °C, and a 5-min final extension at 72 °C. For Afu-22, 23, 57, 58, 62, and 69, the results were identical at both temperatures, 52 and 57 °C. The size of alleles was determined using a 310 DNA sequencing machine (ABI, USA) with internal standards. We observed the number of alleles at each locus and in each population, as well as the allele frequences.

RESULTS

The D-loop (mtDNA)

We succeeded in amplification of D-loop region using DNA extracted from 12 of 44 archival samples of *A. sturio*. Amplification with the sturgeon specific primers resulted in a 250-bp fragment (10 individuals) or a 330-bp fragment (1 individual), including two or three repeats, respectively. In *A. sturio,* the length of the repeated unit was 80 bp, and in *A. o. oxyrinchus*, it was 79 bp. The repeated units of *A. sturio* and *A. oxyrinchus* differed by 11 substitutions and one deletion or insertion, respectively. No heteroplasmy was found in either species.

We observed three different mitochondrial haplotypes in *A. sturio*, and another three haplotypes in *A. o. oxyrinchus*. In *A. sturio*, the most common haplotype was identical to the haplotype found in sturgeon from the Gironde River. The second haplotype was present in an *A. sturio* juvenile artificially reproduced in the 1890s (MfN Berlin, see table I). In this case, a length variation in one of the repeated units was observed. The third *A. sturio* haplotype was found in two individuals: a sturgeon caught in 1823 in the Tiber River, Italy (MNHN 5165; table I), and a sturgeon captured in the Adriatic Sea (MF 7647; table I). This haplotype was differentiated from all other *A. sturio* haplotypes by a single T → C substitution.

We found the same *A. o. oxyrinchus* haplotype in specimens from both the Delaware and Hudson Rivers. It differed from the haplotype found in the St. John River by a single A → G substitution. Also, we observed the third *A. o. oxyrinchus* haplotype in only one specimen from the Delaware River. This haplotype had the same A → G substitution and an additional substitution (C → T) in the 3'-flanking region of the repeated unit.

Genetic distance calculations within *A. sturio* based on the D-loop data showed a high similarity between the North Sea and the Gironde River populations and a more basal position of the Mediterranean and Adriatic Sea populations (table II and figure 1). The genetic distance was 0.0004 between sturgeon from the Mediterranean/Adriatic Sea and the North Sea/Gironde River populations. In *A. o. oxyrinchus,* genetic distances ranged between 0.008 and 0.139. The St. John River and the merged Hudson and Delaware River populations of *A. o. oxyrinchus* had a high genetic similarity. In summary, a high level of genetic similarity was found within both species (table II and figure I).

Table II. p-distance values used for tree calculation calculated in MEGA (Kumar, Tamura and Nei, 1993). Haplotypes are named in relation to their sampling location

	A. sturio		*A. o. oxyrinchus*		
	Gironde River North Sea	Mediterranean Sea Adriatic Sea	Delaware River	Hudson and Delaware Rivers	St. John River
Gironde River, North Sea		0.004	0.135	0.139	0.139
Mediterranean Sea, Adriatic Sea			0.131	0.136	0.136
Delaware River				0.021	0.008
Hudson and Delaware Rivers					0.021
St. John River					

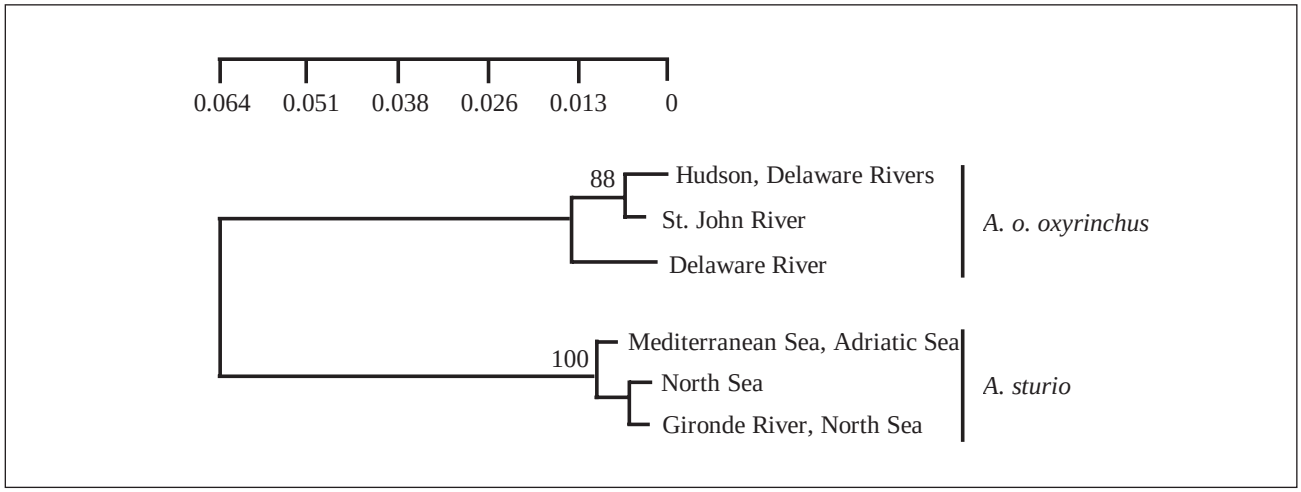

Figure 1. Genetic distance tree using a 250 bp of the mitochondrial D-loop and the $tRNA^{Pro}$ gene basing on the different haplotypes found in this study. The tree was calculated in MEGA (Kumar, Tamura and Nei, 1993) based on p-distance values and neighbour-joining. Bootstrap values > 50 are shown on the branches

Microsatellites

We obtained microsatellites from 38 archival samples of *A. sturio*. The following microsatellites were used for statistical calculations: Afu-19, 34, 39, 54, and 68. The microsatellites Afu-22, 23, 57, 58, 62, and 69 were not included in the analyses since the PCR products were not synthesised or artefacts were formed. The microsatellites Afu-19, 34, 39, 54, and 68 showed polymorphic band patterns in both species, *A. sturio* and *A. o. oxyrinchus*.

In *A. sturio*, five alleles were observed for Afu-19, four for Afu-34, two for Afu-39, three for Afu-54, and four for Afu-68. Allele frequencies of the polymorphic loci in *A. sturio* and *A. o. oxyrinchus*, as well as the differences in the allele frequencies and distribution observed between the North and Baltic Sea specimens of *A. sturio*, are shown in table III. For example, the 121-bp allele of Afu-19 was observed two times: first, in a sturgeon caught in 1910 in the North Sea near the town of Stroemstad, Sweden (NRM 21708), and second, in a sturgeon captured in 1992 in the North Sea off the Dutch coast (Universität Rostock A4). This allele was missing in specimens from the Baltic Sea. On the whole, the following alleles were observed exclusively in the North Sea specimens: the 121 and 124 bp of Afu-19; the 147 bp of Afu-34; the 120 bp of Afu-39; and the 152 bp of Afu-68. On the contrary, the alleles 130 of Afu-19 and 180 of Afu-180 were present exclusively in the Baltic Sea specimens.

Combining the data for the microsatellites, we found 18 alleles in all archival specimens. In contrast, the German broodstock showed only eight alleles (table IV). The following alleles were missing: the 121, 127, and 130 bp of Afu-19; the 140 and 144 bp of Afu-34; the 120 bp of Afu-39; the 180 bp of Afu-54; the 128, 136, and 152 bp of Afu-68. The 136-bp allele of Afu-68, which was missing in the German broodstock, was the most common allele of this locus in archival samples. Possibly, if more specimens from the Gironde River population had

Table III. Allele frequencies of polymorphic loci observed in archival samples of *A. sturio* and in *A. o. oxyrinchus*

		A. sturio		*A. o. oxyrinchus*		
Locus Allele (base pair)		North Sea	Baltic Sea	Delaware R.	Hudson R.	St. John R.
Afu-19		n = 15	n = 9	n = 16	n = 12	n = 60
	118	0.067	0.111	0	0	0
	121	0.133	0	0.062	0.042	0
	124	0.133	0	0.062	0	0.016
	127	0.667	0.778	0.625	0.666	0.742
	130	0	0.111	0	0	0
	145	0	0	0.250	0.292	0.242
Afu-34		n = 15	n = 9	n = 16	n = 12	n = 10
	138	0	0	0.062	0.457	0
	141	0.034	0.111	0.062	0.500	0
	144	0.233	0.167	0.625	0	0.550
	147	0.200	0	0	0	0.050
	150	0.533	0.722	0.125	0	0.400
	153	0	0	0.125	0.042	0
Afu-39		n = 17	n = 7	n = 16	n = 14	n = 60
	120	0.176	0	0.094	0.036	0
	123	0.823	1.000	0.906	0.964	1.000
Afu-54		n = 17	n = 9	n = 16	n = 13	n = 60
	180	0	0.222	0	0	0
	184	0.500	0.611	0.812	0.810	1.000
	188	0.500	0.167	0.156	0.190	0
	192	0	0	0.031	0	0
Afu-68		n = 17	n = 9	n = 16	n = 12	n = 59
	132	0	0	0.031	0	0
	136	0.794	0.833	0.094	0.208	0
	140	0.148	0.111	0.125	0.458	0.364
	144	0.029	0.056	0.281	0.125	0.220
	148	0	0	0.031	0	0.102
	152	0.029	0	0.219	0.208	0.144
	156	0	0	0.156	0	0.017
	160	0	0	0.062	0	0.152

been included in our study, more alleles would have been found within this population.

The length of the microsatellite alleles in *A. o. oxyrinchus* was in the same range as in *A. sturio*. Frequencies of all alleles are shown in table IV. The number of alleles ranged from 14 (Hudson and St. John Rivers) to 22 (Delaware River) (table IV). Specifically, the highest number of alleles was observed for Afu-68 (n = 8), followed by Afu-34 (n = 5).

The following alleles were found exclusively in *A. sturio*: the 128 bp of Afu-68; the 180 bp of Afu-54; and the 118 and 130 bp of Afu-19. The alleles 138 and 153 of Afu-34; 192 of Afu-54; and 132, 148, 156, and 160 of Afu-68 were present in *A. o. oxyrinchus,* but not in *A. sturio*.

DISCUSSION

Analyses of archival samples

Until now, the only analyses of genetic variability at the population or subpopulation level among fishes using archival samples have been performed on the Atlantic salmon *Salmo salar* L., 1758 (Nielsen, Hansen and Loeschcke, 1997, 1999). Therefore, ours is the first detailed research on genetic variability of extinct sturgeon stocks. Previously, Ferguson and Duckworth (1997) studied only a few *A. fulvescens* specimens from a museum collection in London. However, our results on DNA extraction and amplification were similar to those of Nielsen, Hansen and Loeschcke (1997), who had an extraction and amplification rate in the case of archival samples of the Atlantic salmon of more than 90 %. The extraction and amplifica-

Table IV. Distribution of alleles observed in *A. sturio* and *A. o. oxyrinchus*. (1): The broodstock kept at the Institute of Freshwater Ecology and Inland Fisheries (Berlin) originated from artificial reproduction of two *A. sturio* individuals caught in the Gironde River basin in France (Williot *et al.*, 1997)

	A. sturio		*A. o. oxyrinchus*		
	Archival samples 1823-1996	Broodstock (Berlin)[1]	Delaware River	Hudson River	St. John River
Locus	n = 38	n = 27	n = 16	n = 14	n = 60
Afu-19	118, 121, 124, 127, 130, 145	118, 145	121, 124, 127, 145	121, 127, 145	124, 127, 145
Afu-34	141, 144, 147, 150	147, 150	138, 141, 144, 150, 153	138, 141, 153	144, 147, 150
Afu-39	120, 123	123	120, 123	120, 123	123
Afu-54	180, 184, 188	184, 188	184, 188, 192	184, 188	184
Afu-68	128, 136, 140, 152	140	132, 136, 140, 144, 148 152, 156, 160	140, 144, 148, 156	140, 144, 148, 152, 156, 160
Overall loci	19	8	22	14	14

tion rate for archival samples of *A. sturio* in our study was 25 % for mtDNA, and about 86 % for microsatellites.

Our data support the statement of Nielsen, Hansen and Loeschcke (1997) that archival samples are useful sources of information for tracking genetic changes in fish populations, and are therefore valuable for conservation and restoration programmes. The analysed archival samples provided us with a unique opportunity to review the historical structure of the gene pool of *A. sturio* and the dynamics of its change.

Genetic variability and conservation aspects

Sturgeon from the Delaware and Hudson River populations are part of a mixed-stock of *A. o. oxyrinchus* that is located along the North American Atlantic coast and reproduces mainly in the Hudson River (Waldman, Hart and Wirgin, 1996; Waldman and Wirgin, 1998; Secor and Waldman, 1999). The existence of native Delaware River individuals is questionable. However, the large number of alleles that we found in specimens from the Delaware River population points to possible crosses between different strains or to a hybrid origin of this stock. It is doubtful that the small number of alleles observed in specimens from the St. John River population reflects the entire genetic variability in this population: specimens analysed in this study resulted from artificial reproduction of a limited number of breeders. The inclusion of more specimens would probably result in a higher number of microsatellite alleles in sturgeon from the St. John River population. Our data on the analysis of mtDNA support this assumption. We detected only one mitochondrial haplotype in the St. John River sturgeon, and two haplotypes in individuals from the Delaware/Hudson population.

In contrast to Miracle and Campton (1995), but in agreement with Brown, Beckenbach and Smith (1992) and Brown *et al.* (1996), we observed no heteroplasmy in *A. o. oxyrinchus*. Brown *et al.* (1996) described one specimen of *A. o. oxyrinchus* showing three repeated units. In our study, we found a single *A. sturio* showing three repeated units. However, a very low rate of length variation and the absence of heteroplasmy in *A. sturio* and *A. o. oxyrinchus* indicate a genetically-based mechanism of restriction which prevents the inclusion or deletion of repeated units described in other American sturgeon species (Buroker *et al.*, 1990; Brown, Beckenbach and Smith, 1992; Brown *et al.*, 1996).

Our analyses of archival samples of *A. sturio* showed a decline in genetic variability between 1823 and 1992. These data illustrate the coincidence between the decline in genetic diversity decrease and periods of rapid decrease in population size or population crashes. Obviously, the drop in population size led to a fixation of particular alleles in the populations of surviving individuals. For example, the 120-bp allele of Afu-39 was observed in only four sturgeon caught between 1870 and 1890 in the Elbe River and the North Sea, as well as in two specimens of unclear origin. One specimen (MNHM 4634) was labelled with the sampling location "Russia before 1870", and the sampling location of the second specimen caught between 1798 and 1806 was unknown (NRM 94). The aforesaid allele has been missing since 1890. The following seven microsatellite alleles were also missing after 1950: the 124, 127, and 130 bp of Afu-19; the 144 bp of Afu-34; the 120 bp of Afu-39; the 180 bp of Afu-54; and the 128 bp of Afu-68. Additionally, the mitochondrial haplotypes found in the Mediterranean and Adriatic Sea populations, as well as one from the North Sea individuals, were not found after 1950, either.

Overfishing and damming of rivers were the most significant factors that caused sturgeon population crashes (Birstein, 1999). Currently, *A. sturio* is on the verge extinction in German waters (Debus, 1995). In the 19th century it was the most economically valuable fish species in Germany (Debus, 1995; Spratte and Rosenthal, 1996). The last catches of *A. sturio* individuals –during the spawning migration of sturgeon into the Eider River, a tributary of the North Sea– were reported in 1969 (Spratte and Rosenthal, 1996).

At present, *A. sturio* inhabits only the Gironde-Garonne-Dordogne River basin in France (Holčík *et al.*, 1989; Birstein, Betts and DeSalle, 1998). Unfortunately, no data are available on the genetic structure of the Gironde River population. The 27 individuals representing the first generation of two sturgeons caught in French waters in the early 1990s had eight of 19 alleles found in the archival samples. These individuals represent the broodstock for potential restoration of *A. sturio* in German waters. Therefore, restoration of *A. sturio* in German waters using juveniles produced from

these fish would be based on a maximum of about 42 % of the original genetic variability.

The microsatellite data are a valuable genetic characteristic of a sturgeon broodstock for establishing a breeding programme to safeguard the existing genetic variability. The genetic screening of specimens reared at the Cemagref facility in France and a joint German-French breeding programme are important to increase the chances of a successful restoration of *A. sturio* in German waters, as well as for successful stocking of this species in French waters. It is crucial for the restoration programmes to focus their future genetic analyses on the entire stock of the artificially produced *A. sturio* as well as on the wild individuals caught in the Gironde-Garonne-Dordogne River basin in France.

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